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Isolation of amoenamide A and five antipodal prenylated alkaloids from *Aspergillus amoenus* NRRL 35600

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ABSTRACT

A new prenylated alkaloid, Amoenamide A (**6**), was isolated from the fungus *Aspergillus amoenus* NRRL 35600. Previously, **6** was postulated to be a precursor of Notoamide E4 (**21**) converted from Notoamide E (**16**), which was a key precursor of the prenylated indole alkaloids in the fungi of the genus *Aspergillus*. We previously succeeded in the isolation of two pairs of antipodes, Stephacidin A (**1**) and Notoamide B (**2**), from *A. amoenus* and *A. protuberus* MF297-2 and expected the presence of other antipodes in the culture of *A. amoenus*. We here report five new antipodes (**7**–**11**) along with a new metabolite (**12**), which was isolated as a natural compound for the first time, from *A. amoenus*.

Introduction

We have reported the isolation of biosynthetically interesting prenylated indole alkaloids from three fungi of the genus *Aspergillus*. (+)-Stephacidin A (**1**), (−)-Notoamide B (**2**), and their congeners were isolated from *A. protuberus* MF297-2,[1] and successively the antipodes, (−)-**1** and (+)-**2**, were obtained from *A. amoenus* (formerly *A. versicolor*) NRRL 35600[2] (Scheme 1). Recently, we reported the isolation of seven novel prenylated indole alkaloids, the Taichunamides, along with (+)-6-*epi*-Stephacidin A (**3**) and (+)-Versicolamide B (**4**) from *A. taichungensis* IBT 19404 (Scheme 1).[3] Interestingly, **1**/**2** and **3**/**4** contain a *syn*- and *anti*-bicyclo[2.2.2]diazaoctane cores, respectively (the *syn*- and *anti*-relationship is based on the H21 and bridging amide C18/N19 relative stereochemistry), and these cores are plausibly formed through an intramolecular hetero Diels–Alder reaction from a common precursor, Notoamide S (**5**)[4] (Scheme 1). To date, we have been studying the structures,[1,4–8] syntheses,[9–15] and bioconversions[4,13,15–17] of prenylated indole alkaloids from *A. protuberus* and the structures[2,4] and bioconversions[4,13–15] of those from *A. amoenus*. Curiously, we discovered that *A. amoenus* produced an enantiomeric mixture of **3** enriched with the (−)-isomer.[4] The presence of the enantiomerically pure (+)-**4** in *A. amoenus* suggests that the fungus possesses the oxidase, which selectively converts (+)-**3** into (+)-**4**, but does not process (−)-**3** (Scheme 1).[4] Successively, we have been studying the structures of metabolites from *A. amoenus* and here report the isolation of a new prenylated alkaloid, Amoenamide A (**6**), five new antipodes (**7**–**11**), and a new metabolite (**12**), which was isolated as a natural compound for the first time (Fig. 1).

Results and discussion

The fungus, *A. amoenus* NRRL 35600, was cultured on rice at 25 °C for a month and the metabolites were extracted with *n*-BuOH. After solvent partition, the metabolites were purified by column chromatography and HPLC to yield a new compound, Amoenamide A (**6**), five new antipodes, (−)-Notoamides F (**7**),[6] I (**8**),[6] R (**9**),[8] and U (**10**),[18] and (+)-Notoamide L (**11**),[7] and a new natural compound, (−)-6-*epi*-Notoamide I (**12**),[17] and fourteen known alkaloids, (−)-Stephacidin A (**1**), (+)- and (−)-6-*epi*-Stephacidin A (**3**), (+)-Versicolamide B (**4**), (+)-Notoamides A (**13**)[1] and B (**2**),[1] Notoamides C (**14**),[1] D (**15**),[1] E (**16**),[5] M (**17**),[7] Q (**18**),[8] and S (**6**),[4] Dehydronotoamide C (**19**),[11,19] and Speramide B (**20**)[20] (Fig. 1).[21]

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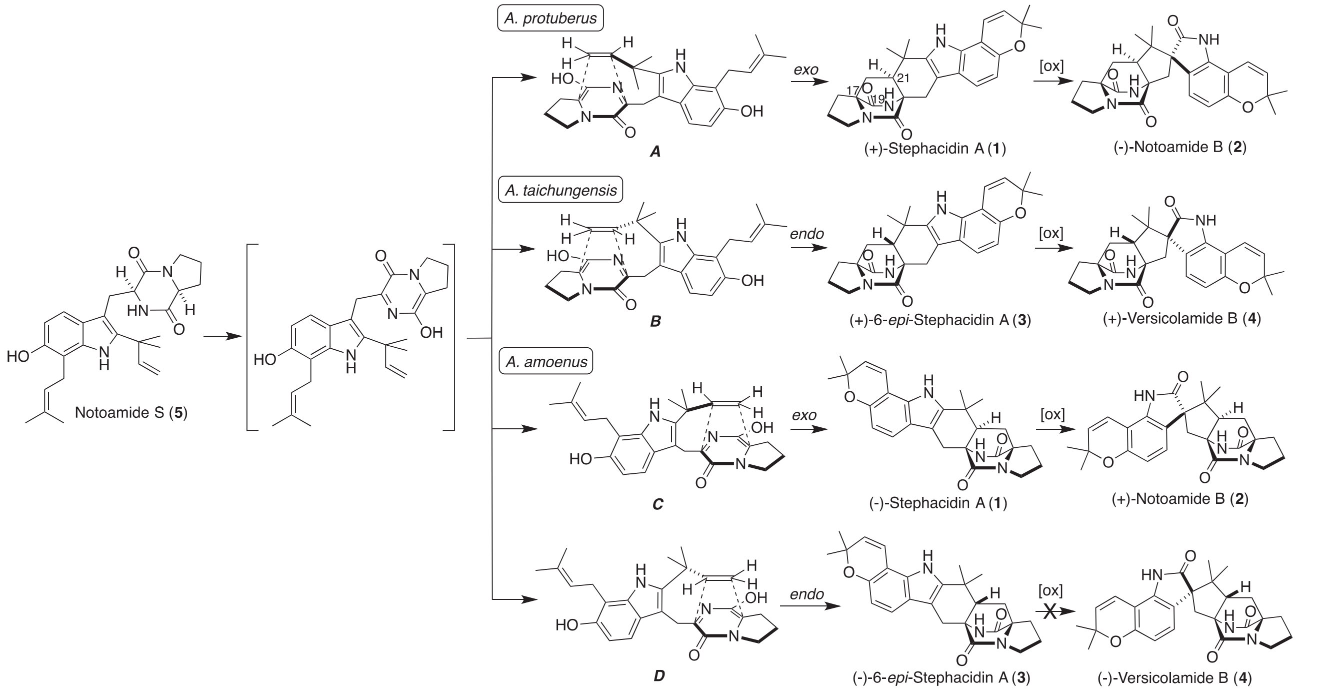


Scheme 1. Proposed facial specificities of intramolecular hetero Diels–Alder reactions for major metabolites in three species, *A. protuberus*, *A. taichungensis*, and *A. amoenus*.

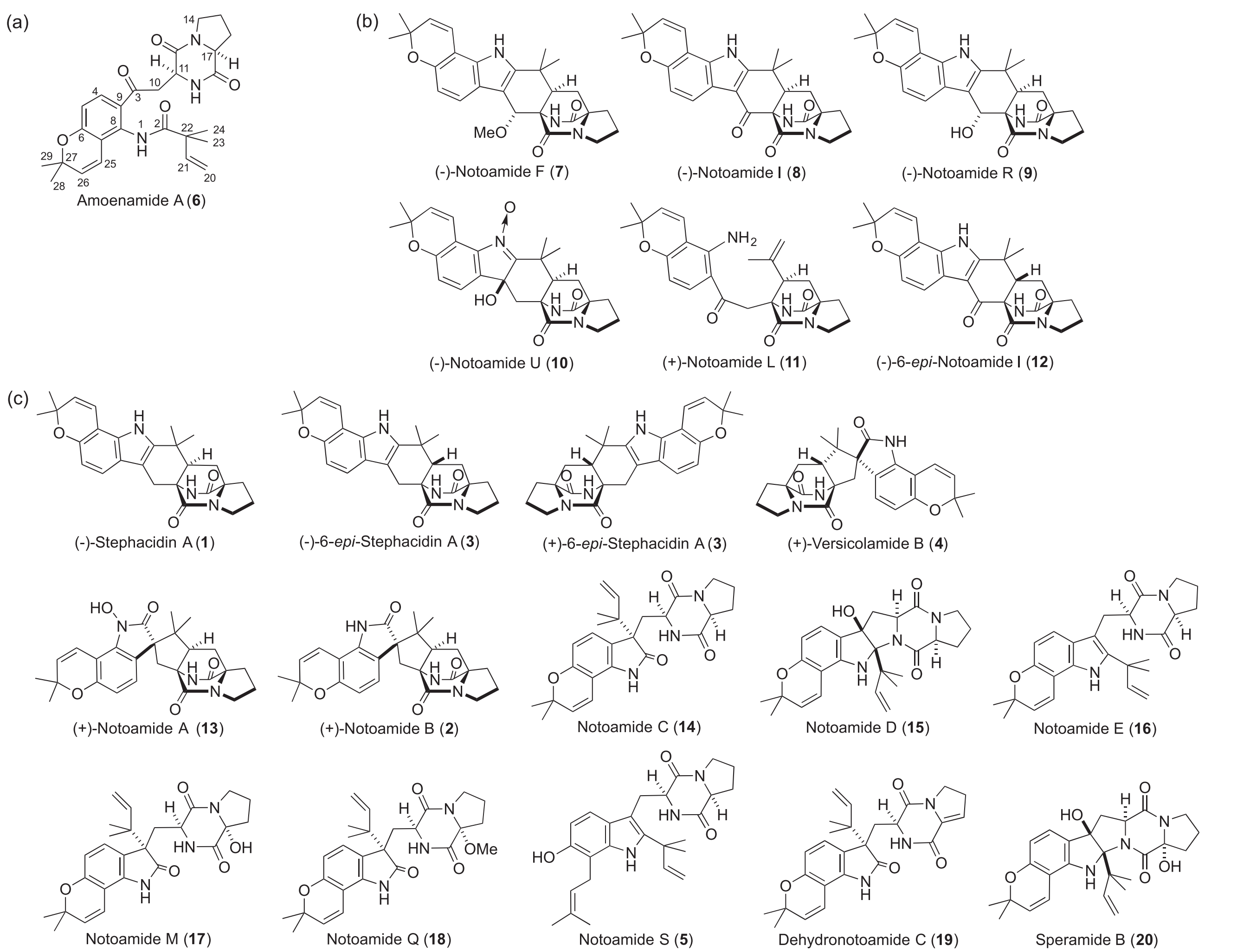


Fig. 1. Structures of (a) a new compound, amoenamide A (**6**), (b) five antipodes (**7–11**) and a new natural compound (**12**), and (c) fourteen known compounds (**1–5** and **13–20**).

Table 1
^{1}H and ^{13}C NMR data for **6** in DMSO-d_6.

Position	δ_C	δ_H (J in Hz)	HMBC
1		9.57 s	2, 7, 9
2	174.7		
3	198.1		
4	129.8	7.63 d 8.6	3, 6, 8
5	112.7	6.73 d 8.6	6, 7, 9
6	155.7		
7	117.4		
8	132.9		
9	126.5		
10	39.9	3.04 dd 6.0, 17.7	3, 11, 12
		3.50 dd 6.0, 17.7	3, 11, 12
11	50.7	4.57 t 6.0	3, 10, 12
12	165.7		
14	44.2	3.33 m	
		3.40 m	
15	21.8	1.82 m	
		1.86 m	
16	27.5	1.90 m	
		2.15 m	
17	58.0	4.26 t 7.8	16, 18
18	169.5		
19		7.93 s	11, 12, 17
20	113.6	5.16 dd 1.0, 10.4	22
		5.22 dd 1.0, 17.9	21, 22
21	142.4	6.13 dd 10.4, 17.9	22, 23, 24
22	45.1		
23	23.6	1.29 s	2, 21, 22, 24
24	23.6	1.29 s	2, 21, 22, 23
25	118.1	6.14 d 9.7	6, 8, 27
26	129.9	5.78 d 9.7	7, 27
27	76.5		
28	27.0	1.39 s	26, 27, 29
29	27.0	1.40 s	26, 27, 28

The molecular formula of **6** was determined to be $C_{26}H_{31}N_3O_5$ by HRESIMS. The ^{1}H NMR spectrum (DMSO-d_6) (Table 1) showed four doublet olefinic and aromatic protons (δ 5.78 (d, J = 9.7 Hz, H-26), 6.14 (d, J = 9.7 Hz, H-25), 6.73 (d, J = 8.6 Hz, H-5), and 7.63 (d, J = 8.6 Hz, H-4)), a monosubstituted double bond (δ 5.16 (dd, J = 1.0, 10.4 Hz, H-20), 5.22 (dd, J = 1.0, 17.9 Hz, H-20), and 6.13 (dd, J = 10.4, 17.9 Hz, H-21)), two exchangeable protons (δ 7.93 (s, H-19) and 9.57 (s, H-1)), two methine protons (δ 4.26 (t, J = 7.8 Hz, H-17) and 4.57 (t, J = 6.0 Hz, H-11)), and four singlet methyl groups (δ 1.29 (6H, s, H_3-23 and H_3-24), 1.39 (3H, s, H_3-28), and 1.40 (3H, s, H_3-29)), which indicated that **6** was a congener of the Notoamides. The analysis of 2D NMR spectra, including COSY, HMQC, and HMBC, showed three substructures, a 5,6-disubstituted 2,2-dimethyl-2*H*-chromene (A), a 3-substituted hexahydropyrrolo[1,2-*a*]pyrazine-1,4-dione (B), and a 3-substituted 3-methylbut-1-ene (C) (Fig. 2a). Key HMBC correlations showed the substructure C was connected to C-8 of the substructure A through an amide group (δ_H 9.57 (H-1), δ_C 174.7 (C-2)) (Fig. 2b). The substructure B was connected to C-9 of the substructure A through a ketone group (δ_C 198.1 (C-3)). The 11*S*,17*S*-configuration for **14** were determined by a NOE correlation and chemical degradation,[1] and from the biogenetic relationship with **14**, the absolute configuration of **6** was indicated as 11*S*,17*S*. Thus, the structure of **6** was established.

Previously, we proposed that Notoamide E (**16**) would be a key biosynthetic intermediate for the Notoamides and Stephacidin A (**1**) in *A. protuberus*. In order to confirm this proposal, we performed bioconversion of ^{13}C-labeled **16**.[5] In this experiment, a new compound, Notoamide E4 (**21**), was obtained as a metabolite and we proposed a *N*-formylkynurenine derivative corresponding to **6**, was a putative precursor of **21** (Scheme 2). In the present work, we isolated natural **6** from the fungal culture, the presence of which strongly supports our hypothesis (Scheme 2).

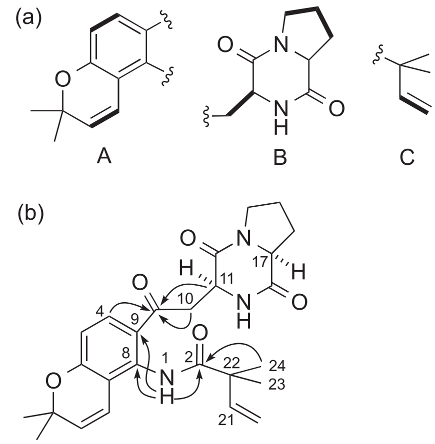
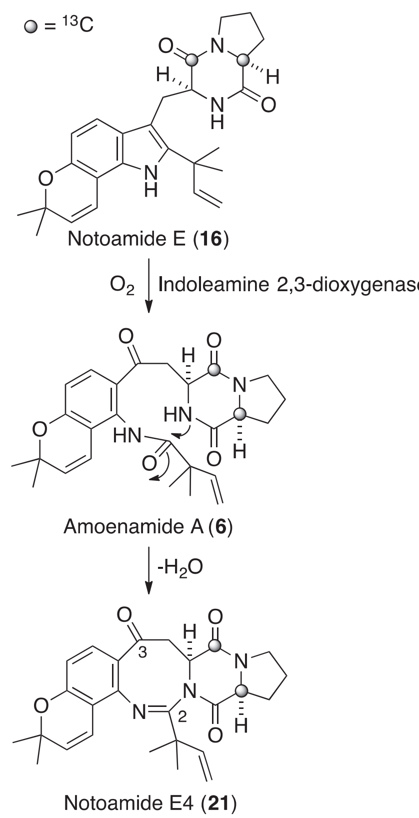


Fig. 2. (a) Substructures A–C indicated by COSY (bold lines), HMQC, and HMBC spectra and (b) key HMBC correlations for **6**.



Scheme 2. Possible biosynthetic pathway from **16** to **21**.

After the isolation of the antipodes of Stephacidin A (**1**) and Notoamide B (**2**) from *A. protuberus* MF297-2[1] and *A. amoenus* NRRL 35600[2] as major metabolites, the presence of other antipodal metabolites in *A. amoenus* has also been expected to date. Herein, we succeeded in the isolation of the antipodes of previously reported natural alkaloids namely, (−)-Notoamides F (**7**),[6] I (**8**),[6] R (**9**),[8] and U (**10**),[18] and (+)-Notoamide L (**11**),[7] from *A. amoenus*. In addition, (−)-6-*epi*-Notoamide I (**12**) was isolated as a natural compound for the first time, although (±)-**12** was obtained by the bioconversion of (±)-6-*epi*-Notoamide T in *A. protuberus* MF297-2.[17] The elucidation of the biochemical basis for the stereochemical diversity of these families of prenylated indole alkaloids biosynthesized within orthologous species of *Aspergillus* fungi, specifically *A.*

protuberus MF297-2, *A. amoenus* NRRL 35600, and *A. taichungensis* IBT 19404 is ongoing in our laboratories.[22–24]

Acknowledgments

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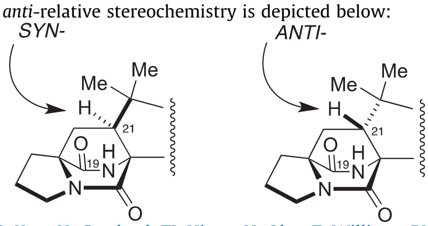
(b) *syn*- and *anti*-relative stereochemistry is depicted below:



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21. The fungus, *A. amoaenus* NRRL 35600, was obtained from the basidioma of *Ganoderma australe* collected in a Hawaiian forest. The fungus was cultured on rice media (100 g × 50) in Erlenmeyer flasks (500 mL) at 25 °C for a month. The metabolites were extracted with *n*-BuOH and the concentrated aqueous solution was extracted with *n*-BuOH. The *n*-BuOH solution was evaporated and the dried material was partitioned between *n*-hexane and 90% MeOH/H_2O. The 90% MeOH/H_2O fraction (14.8 g) was subjected to ODS chromatography with 75% MeOH/H_2O to yield three fractions (fractions A (2.5 g), B (2.0 g), and C (1.1 g)) containing the prenylated indole alkaloids. Fraction A was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (10:19:1) and then NH_2 chromatography with CH_2Cl_2/MeCN (1:1 and 1:3) and MeCN/H_2O (1:1) followed by HPLC (phenyl-hexyl (MeOH/H_2O) and gel filtration (MeOH)) to afford (−)-**1** (4.9 mg), (+)-**4** (0.4 mg), (−)-**8** (0.7 mg), (−)-**10** (0.5 mg), (+)-**11** (0.8 mg), and **18** (8.4 mg). Fraction B was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (10:19:1) and then NH_2 chromatography with CH_2Cl_2 and CH_2Cl_2/MeCN (3:1) followed by gel filtration HPLC (MeOH) to afford **5** (17.1 mg), (−)-**12** (0.4 mg), **15** (46.9 mg), **19** (0.9 mg), and **20** (29.9 mg). Fraction C was purified by SiO_2 chromatography with *n*-hexane/CH_2Cl_2/MeOH (30:19:1 and 10:19:1) followed by HPLC (phenyl-hexyl (MeOH/H_2O), NH_2 (CH_2Cl_2/MeCN), and gel filtration (CH_2Cl_2/MeOH/H_2O)) to afford (+)-**2** (2.2 mg), (+)-**3** (0.12 mg), (−)-**3** (0.29 mg), **6** (1.1 mg), (−)-**7** (1.7 mg), (−)-**9** (0.5 mg), (+)-**13** (1.1 mg), **14** (1.6 mg), **16** (2.1 mg), and **17** (0.3 mg).Amoenamide A (**6**): $[\alpha]_D^{20}$ −6.0° (*c* 0.91, MeOH); UV (MeOH) λ_{max} (log ε) 308 (3.04), 252 (3.56), 206 (4.90) nm; IR (film) ν_{max} 3356, 2925, 2855, 1674, 1460, 1117 cm^{-1}; HRESIMS *m/z* 488.2183 $[M+Na]^+$ (calcd for $C_{26}H_{31}N_3O_5Na$, 488.2156); ^1H and ^{13}C NMR data (DMSO-d_6), see Table 1.(−)-Notoamide F (**7**): $[\alpha]_D^{20}$ −12° (*c* 1.4, MeOH); (+)-**7**: $[\alpha]_D^{21}$ +1.9° (*c* 0.27, MeOH).[6](−)-Notoamide I (**8**): $[\alpha]_D^{20}$ −58° (*c* 0.46, MeOH), $[\alpha]_D^{24}$ −69° (*c* 0.10, MeOH/$CHCl_3$ 1:1); (+)-**8**: $[\alpha]_D^{29}$ +31° (*c* 0.1, MeOH/$CHCl_3$ 1:1).[6](−)-Notoamide R (**9**): $[\alpha]_D^{20}$ −44° (*c* 0.19, MeOH); (+)-**9**: $[\alpha]_D^{14}$ +38° (*c* 0.5, MeOH).[8](−)-Notoamide U (**10**): $[\alpha]_D^{20}$ −44° (*c* 0.23, MeOH); (+)-**10**: $[\alpha]_D^{25}$ +54.1° (*c* 0.1, MeOH).[18](+)-Notoamide L (**11**): $[\alpha]_D^{20}$ +21° (*c* 0.17, MeOH); (−)-**11**: $[\alpha]_D^{23}$ −17° (*c* 0.77, MeOH).[7](−)-6-*epi*-Notoamide I (**12**): $[\alpha]_D^{20}$ −52° (*c* 0.48, MeOH).
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